Molecular Features of Modification Clusters in the Anticodon Loop of tRNA: A Quantum Mechanical and Molecular Dynamics Study

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Post-transcriptional hypermodifications to transfer RNA (tRNA) play important roles in supporting the overall tRNA biology by potentially providing structural stability, protecting against several physical, chemical, biological degradations, and enhancing protein synthesis. Sequence analysis shows that many unique tRNA hypermodifications occur at the 37th position. In addition to enhancing interactions between tRNA anticodons and mRNA, hypermodifications at the 37th position have been proposed to increase the stability of the anticodon loop through stronger stacking interactions with the neighboring nucleobases at positions 36 and 38. However, information about distinct nucleobase–nucleobase interactions is difficult to obtain through experimental studies.

This study aims to use computational chemistry to provide the missing structural and energetic information about the impact of a range of bulky hypermodifications on the magnitude of nucleobase–nucleobase stacking interactions. Specifically, three hypermodified base families known to be present at the 37th position of tRNA were considered including the parent i^{6}A (N6-isopentenyladenosine), t^{6}A (N6-threonyl carbamoyladenosine), and yW (wybutosine) modifications, as well as their derivatives formed through the incorporation of additional chemical substituents (such as a hydroxy, methyl, thiomethyl or peroxy group). Initially, different conformations of the modifications were optimized using density functional theory (DFT) calculations (B3LYP-D3(BJ)/6-311++G(2df,2p)//B3LYP-D3(BJ)/6-311+G(2df,p)). The lowest energy conformers were then used to determine the B3LYP-D3(BJ)/6-311+G(2df,p) stacking interactions between the hypermodified bases and the natural RNA nucleobases (A, C, G, and U) by scanning the dimer potential energy surfaces as a function of the vertical displacement between the monomers (R_1), their relative angle of rotation (θ), and the horizontal displacement of one monomer with respect to the other (R_2).

Our data emphasizes that most hypermodifications lead to an increase in the strength of non-covalent interactions, but through different mechanisms. Specifically, some hypermodifications change the stacking potential of the nucleobase ring, while others enhance the stacking interactions through the formation of additional secondary interactions involving the bulky moiety. Interestingly, the increase in the strength of the interactions is dependent on the identity of the neighboring nucleobase and whether the 5' or 3' neighboring nucleobase is considered, as well as the orientation of the bulky moiety. This suggests that the impact of the hypermodifications on the structure and stability of the ASL of tRNA arises for several different reasons. Together, these calculations yield an enhanced appreciation of the fundamental chemistry of the tRNA hypermodified bases, which provides clues regarding their functionality, including insight into the roles of the modifications in preventing human diseases.